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Sperm Behavior in the Micropyle of the Medaka Egg

Sumio Ishijima, Yukihisa Hamaguchi and Takashi Iwamatsu[1]

Biological Laboratory, Faculty of Science, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo 152 and [1]Department of Biology, Aichi University of Education, Kariya 448, Japan

ABSTRACT—Sperm behavior in the micropyle of the medaka egg was examined using videomicroscopy. Spermatozoa swimming near the egg, whose progressive speed was similar to that of spermatozoa freely swimming in a medium, entered the micropylar canal after being trapped in the funnel-shaped micropylar vestibule or entered directly. The spermatozoa stalled in the micropylar vestibule entered the micropylar canal with a time lag of less than 0.5 sec. Proceeding at a fairly high speed for a short time, the spermatozoa gradually slowed down when the flagellar movement of the spermatozoa became obstructed by the surface of the micropylar canal, finally reaching the plasma membrane. The spermatozoa which directly entered the micropylar canal maintained progressive speed until their flagellar movement became obstructed by the surface of the micropylar canal. They reached the plasma membrane through the micropylar canal in the same manner as the spermatozoa experiencing collision with the surface of the micropylar vestibule.

INTRODUCTION

In teleost fish, spermatozoa can reach the egg's plasma membrane only by means of a narrow channel, the micropyle of the vitelline envelope. Since the base of the micropyle is as wide as the diameter of the sperm heads, spermatozoa must pass in single file through the micropyle. This has been thought to be the mechanism for blocking polyspermy [2] because medaka eggs do not show a rapid electrical block to polyspermy [6].

Since observation of sperm entry into the micropyle is difficult, little is known about the exact behavior of spermatozoa in the micropyle. Recent videomicroscopy and the transparency of the medaka egg enabled us to record on videotape the movement of the sperm head in the micropyle of the medaka eggs.

The purpose of the present study is to record and analyze the behavior of spermatozoa in the micropyle of the medaka egg. On the basis of this data, the mechanism for blocking polyspermy in the medaka egg is discussed.

MATERIALS AND METHODS

Medaka oocytes and spermatozoa were prepared by the method described earlier [4] and were kept in Ringer's solution (6.5 g NaCl, 0.4 g KCl, 0.113 g $CaCl_2$ and 0.15 g $MgSO_4 \cdot 7H_2O$ per liter of deionized water, pH=7.3 adjusted with $NaHCO_3$) before use. In each experiment, the gametes were freshly prepared and used within 30 min. The observation chamber was constructed by gluing two strips of silicon rubber (1 mm thick) onto a glass slide parallel to each other. An egg was put in the chamber with a small amount of Ringer's solution, and the chamber was covered with a coverslip after removal of the Ringer's solution around the egg. To observe an oblique view of the egg micropyle, the egg was rotated by sliding the coverslip. Sperm movement was observed using a Nikon Optiphot microscope equipped with a plan 40× objective, 10× eyepieces and a National video camera (WV-1300A, Matsushita Communication Industrial Co., Ltd, Yokohama, Japan). While observing the egg displayed on a

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National TN-96 monitor (Matsushita Communication Industrial Co., Ltd), approximately 0.1 ml of sperm suspension was introduced into the observation chamber from an open end. The image was recorded on video tape with a Panasonic video tape recorder (NV-FS900, Matsushita Communication Industrial Co., Ltd).

For detailed field-by-field analysis, images of the head were traced from the video monitor onto transparent plastic sheets using a fine-point marker. The progressive speed of free-swimming spermatozoa was determined by measuring the distance between the positions of the sperm head at the beginning and the end of a time interval.

RESULTS AND DISCUSSION

Movement characteristics of medaka spermatozoa

Medaka spermatozoa swam at a maximum transverse displacement of the sperm flagella of 5.14± 1.73 μm (mean±S.D., n=16) while rotating about their longitudinal axis at a rate of 3.77 per second as previously reported by Ishijima *et al.* [3]. The progressive speed of medaka spermatozoa swimming near the micropyle was 74.0±14.6 μm/sec (mean±S.D., n=19), whereas that of spermatozoa freely swimming in the medium was 74.9± 31.3 μm/sec (mean±S.D., n=25). No difference was found between them, suggesting that sperm movement, especially progressive speed, is not stimulated by the presence of the micropyle.

The progressive speed of medaka spermatozoa was low as compared to that (191.4 μm/sec) of sea urchin spermatozoa [1], whose flagellum is similar in size to that of medaka spermatozoa. The progressive speed is roughly proportional to the square of the maximum transverse displacement of the flagellum. The ratio of the progressive speed to the square of the maximum transverse displacement of the flagellum does not differ between medaka (2.8; maximum transverse displacement of 5.14 μm) and sea urchin spermatozoa (3.0; maximum transverse displacement of 8.0 μm) [1]. This suggests that the low progressive speed of medaka spermatozoa is mainly due to the narrow amplitude of their flagellar waves. The small transverse displacement of the beating flagella of medaka spermatozoa may be critical for sperm progress in the small diameter of the micropyle (see below).

Progressive speed of medaka spermatozoa in the micropyle

Most spermatozoa attached to the surface of the micropylar vestibule, stayed there for approximately 424 msec (S.D.=243 msec, n=20) and then entered the micropylar canal, although some spermatozoa entered directly. Both the progressive speed of the medaka spermatozoa and the size of the micropyle of the medaka egg varied; the micropyle had a mean diameter (d_v) of 5.30 μm (S.D.=1.25 μm, n=21) at the end of its vestibule and a mean diameter (d_c) of 3.19 μm (S.D.=0.84 μm, n=21) at the base of the micropylar canal (Fig. 1). Thus, the profile of the progressive speed of medaka spermatozoa in the micropyle varied. In general, three phases in the time course of the progressive speed were recognized (Fig. 2). The first phase was a decrease in the progressive speed of the spermatozoa from the free-swimming speed

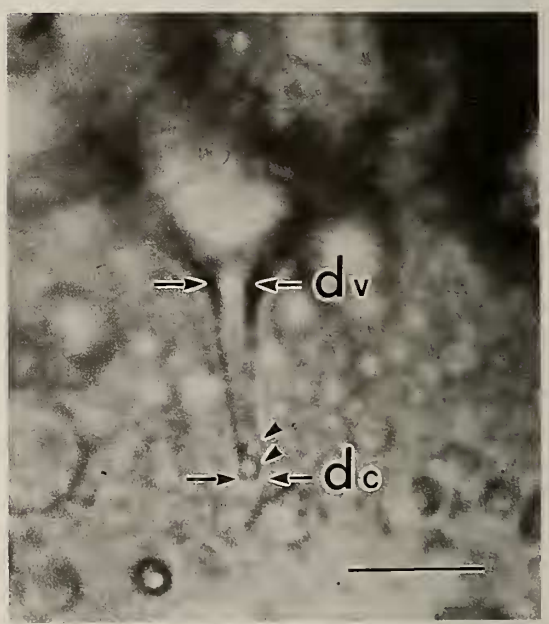

Fig. 1. A phase-contrast videomicrograph of the micropyle of the medaka egg. d_v, diameter of the micropylar vestibule at its base; d_c, diameter of the micropylar canal at its end. Two spermatozoa line up along the micropylar canal (arrowheads). Bar, 20 μm.

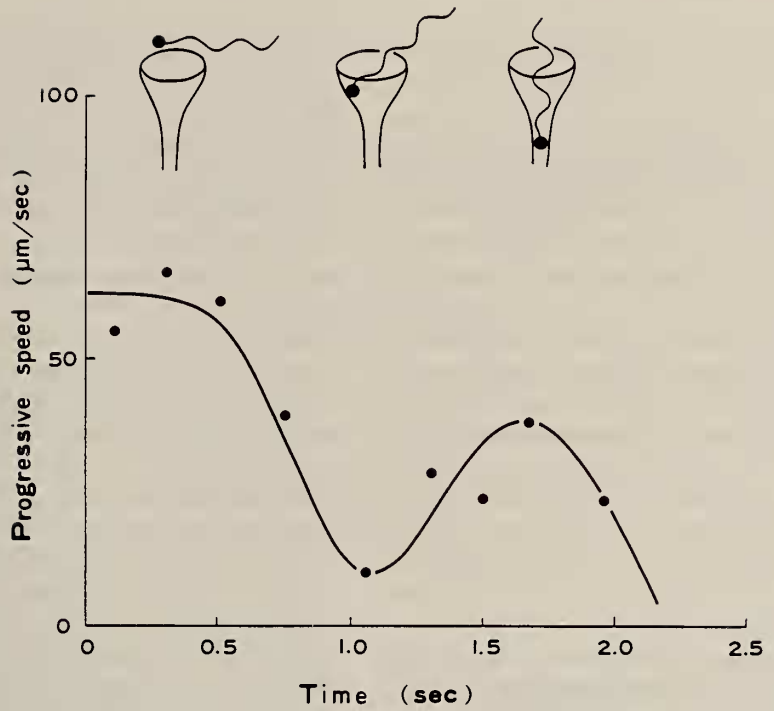

FIG. 2. A typical profile of the time course of the progressive speed of medaka spermatozoa in the micropyle.

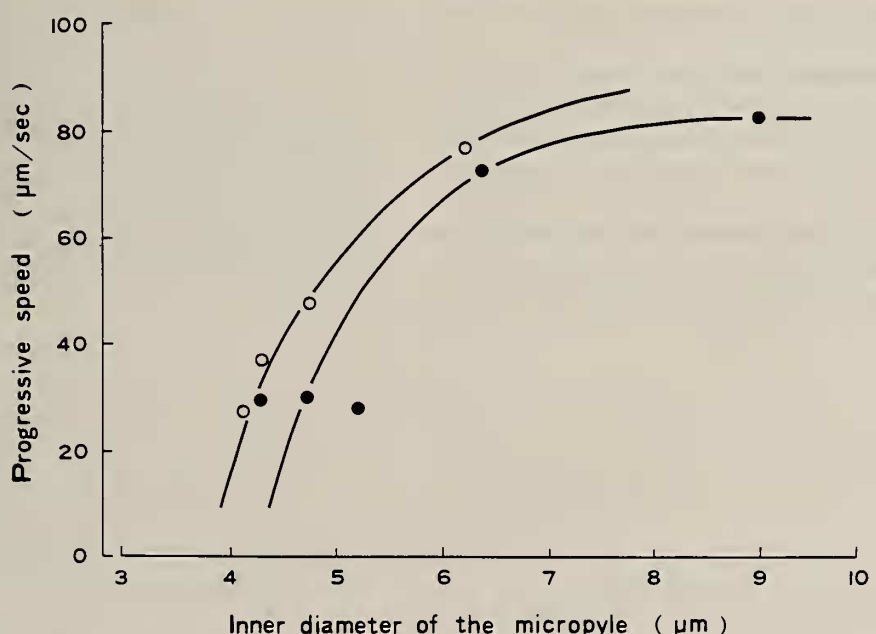

FIG. 3. The relationship between the progressive speed and the diameter of the micropyle. Two examples are shown.

to nearly zero; the spermatozoa which swam freely were trapped in the micropylar vestibule and wandered through it or ceased any progress for less than 0.5 sec as mentioned above. The second phase was the recovery of the progressive speed; the spermatozoa entered the micropylar canal and proceeded without obstruction from the micropylar canal. The final phase was the decrease of the progressive speed until it was reduced to zero; the spermatozoa made their way through the micropyle because the flagellar movement of the spermatozoa was mechanically inhibited by the surface of the micropylar canal, and finally reached the egg's plasma membrane. The inhibitory effect of the micropyle on the progressive speed is shown in Fig. 3. Progressive speed rapidly decreased with a decrease in the diameter of the micropyle. It took 689 ± 428 msec ($n=16$) for the spermatozoa to pass through the micropylar canal (the second and third phases).

No difference was found in the time course of the spermatozoa secondarily entering the micropyle compared to that of the first (data not shown).

Most spermatozoa were trapped in the micropylar vestibule before entering the micropylar canal, suggesting that the micropylar vestibule is very useful in capturing the spermatozoa.

In sperm-egg interaction, there are various mechanisms blocking polyspermy [2]. In medaka, the micropyle plays an important role in blocking polyspermy because only one spermatozoon reached the plasma membrane in our study. The blocking of the polyspermy with the micropyle is probably due to the mechanical inhibition of sperm progression of the surface of the micropyle. The mean diameter of the micropyle at its end ($3.19 \pm 0.84\ \mu m$) is almost the same as that of the head of spermatozoa ($3.71 \pm 0.60\ \mu m$, $n=17$), so that sperm must pass in single file through the micropyle. The decrease in progressive speed (Figs. 2 and 3), especially slow progression of further sperm, may contribute to the effective polyspermy block. There was no difference in sperm behavior in the micropyle between the spermatozoa entering immediately or later. Many spermatozoa could pass through the micropyle which had been isolated from the medaka egg (data not shown). These observations suggest that substances inhibit sperm behavior are not secreted by the plasma membrane and the micropyle.

Most medaka spermatozoa swimming freely in the medium rolled in a clockwise direction on their longitudinal axis when an observer viewed the cell from the anterior ends although some spermatozoa rolled in the other direction [3]. In contrast, the sense of the micropyle is counterclockwise [5]. Therefore, it is unlikely that the chirality of the micropyle is involved in sperm movement when passing through the micropyle.

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